Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Microencapsulation of Lactobacillus acidophilus La_5 at Sodium Alginate and Sodium Caseinate Matrix and its viability under Simulated Gastrointestinal Conditions

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ARTICLE INFO

Article History:

Received:2024/7/21

Accepted:2024/9/11

Keywords:

Microencapsulation,

Probiotic,

Lactobacillus acidophilus,

Sodium alginate,

Sodium caseinate

DOI: 10.22034/FSCT.22.159.144.

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ABSTRACT

The viability of probiotics in sensitive environments, particularly gastrointestinal conditions, is of significant importance. In this study, we used microencapsulation to increase the viability of probiotics Lactobacillus acidophilus La5 under simulated gastrointestinal conditions. These bacteria were microencapsulated using the emulsification method in a matrix of Sodium alginate and Sodium caseinate at five concentration levels, individually and in combination. Then it was examined for viable cell count, encapsulation yield, survival against bile salts, and viability against gastric acid and intestinal fluid with and without bile salts. Our results showed significant differences between the treatments in all tests when comparing the average data ($p<0.05$). The survival of free cells in digestive conditions decreased sharply; however, microencapsulation acted as a protective role, and the survival of microencapsulated strains was higher than that of free cells. The results showed that microencapsulation acts as a protective mechanism for improving the viability of microencapsulated strains compared to free cells. On the other hand, the combination of Sodium alginate and Sodium caseinate as an encapsulant can significantly increase the bacteria's resistance to digestive conditions ($p<0.05$). Among the treatments, the free cells (L-FC) treatment showed the lowest survival against the simulated digestive environment. In the viability test in the intestinal environment with bile salt, no live cells were present after 300 minutes. However, in contrast, the treatment 75% Sodium alginate + 25% Sodium caseinate ($L\text{-}SA_3SC_1$), had the highest encapsulation yield and exhibited the best protective effect against bile salts, gastric acid, and intestinal fluid. In conclusion, microencapsulation using the emulsification method with a combination of Sodium alginate and Sodium caseinate effectively enhances the survival of Lactobacillus acidophilus, thus having potential beneficial effects on human health, particularly in reducing gastrointestinal diseases.

[DOI: 10.22034/FSCT.22.159.144]

1-Introduction

Probiotics are essential beneficial live microbial supplements that, when present in adequate amounts 10^8-10^9 CFU/g in food work in synergy with prebiotics to improve human intestinal function [1, 2]. Probiotics are used in the production of both fermented and non-fermented probiotic food products, dairy and non-dairy products, probiotic pharmaceuticals, dietary supplements, and animal feed [3, 4]. A food product containing a sufficient quantity of live probiotic bacteria can alter the gut microbial flora and provide antioxidant benefits for brain and nervous system health. It can also treat digestive disorders, such as irritable bowel syndrome, inflammatory bowel disease, diabetes, and diarrhea, by competing with pathogens for adhesion to the intestinal mucosa and increasing bacteriocin and organic acid production to lower pH levels [5]. Additionally, probiotics stimulate appetite and improve host nutrition by producing vitamins, eliminating toxins in the diet, and breaking down digestible particles [6]. Most probiotics used in food, dietary supplements, and pharmaceuticals belong to the family of lactic acid bacteria, commonly Lactobacillus and Bifidobacterium genera [6]. Lactobacillus acidophilus (La5) is a commercially used strain in dairy products, infant food, and dietary supplements, known for its probiotic effects. It has antibiotic-like activity, and the secreted molecules in the Lactobacillus acidophilus environment exhibit time-dependent bactericidal effects against pathogens such as Salmonella typhimurium, Listeria monocytogenes, Enteropathogenic Escherichia coli, and Helicobacter pylori [7].

Probiotics in the gastrointestinal tract are influenced by factors such as pH, acidification due to product fermentation, hydrogen peroxide production, and food storage conditions, particularly temperature [3]. Accordingly, using microencapsulation techniques and incorporating probiotics into a carrier capsule enhances the survival of these bacteria in food products, protects them from stomach conditions, enables their controlled release in subsequent sections of the gastrointestinal tract and at the target site, and increases their effectiveness [3, 8]. The controlled release of bioactive compounds also boosts their efficacy and allows for the determination of optimal dosing [8]. Accordingly, microencapsulation of probiotics is defined as a process of entrapping or encapsulating microorganisms within an appropriate polymer to isolate them from the surrounding environment and protect unstable active compounds (core materials) in various polymeric wall matrices. This facilitates their transfer to the target site and improves product stability [9, 10].

Various techniques are used for the microencapsulation of bioactive compounds in the food industry, which can be classified into chemical methods (such as emulsification and liposome entrapment), physical methods (such as spray drying, spray cooling, extrusion, fluidized bed coating, and electrospinning), and physicochemical methods (such as coacervation and sol-gel encapsulation) [3, 10]. The most common methods for probiotic microencapsulation are emulsification, extrusion, and spray drying. Due to the sensitivity of probiotics to high temperatures and the large particle sizes associated with extrusion and spray drying, these methods are less frequently applied. Therefore, emulsification is considered the most effective technique for probiotic microencapsulation [3, 11, 12]. Emulsification is a chemical embedding method in which the core and wall materials (dispersed phase) are added to vegetable oil (continuous phase), followed by the addition of an emulsifier to form a stable emulsion. Microencapsulation then takes place at the micro-scale with the action of a cross-linking agent [13].

Among the materials used for microencapsulation, only a limited number have been approved as safe coating agents. These primarily include biopolymers such as carbohydrates, proteins, gums, lipids, or their derivatives [14]. Based on previous studies, sodium alginate is the main component used in probiotic microencapsulation due to its high

[DOI: 10.22034/FSCT.22.159.144]

safety, non-toxicity, biocompatibility, excellent gelling properties (temperature- and pH-dependent), ease of bead formation, and digestibility [15-17]. Additionally, sodium alginate degrades at low pH levels, releasing probiotics in gastrointestinal conditions [18]. Thus, sodium alginate is considered one of the best biopolymers for use in microcapsules, offering protection and controlled delivery of probiotics to the intestinal environment [15]. Proteins are also excellent coating materials due to their superior physicochemical and functional properties, including emulsifying capacity, gel formation ability, and film-forming potential, making them widely applicable in the food industry [14]. Sodium caseinate in delivery systems improves emulsion formation and stability by reducing surface tension and forming a protective layer around oil droplets. It also exhibits outstanding functional properties such as thickening, emulsifying, foaming, and thermal stability. Due to the acidic pH in the stomach and neutral pH in the intestine, the pH-dependent behaviour of casein can be beneficial for the controlled release of substances. Additionally, casein can penetrate the plasma membrane in an energy-independent manner, enhancing cellular absorption. The twisted structure of casein makes it susceptible to proteolysis, providing good release by proteolytic enzymes in the digestive tract [19]. Due to the sensitivity of sodium caseinate to pH near its isoelectric point, it may not independently provide the required conditions for encapsulating bioactive compounds, which could negatively affect emulsion stability. Therefore, researchers suggest combining two materials as wall-forming agents to improve performance [20].

The effect of sodium alginate and sodium caseinate as coating agents on the survival of probiotic bacteria, including Lactobacillus strains, under gastrointestinal conditions has been reported in similar studies. Qi et al. (2020) investigated the dual coating of Lactobacillus rhamnosus using sodium alginate and low-density methoxyl pectin or K-carrageenan under simulated gastrointestinal conditions and found that the coating process had a positive effect on enhancing probiotic survival [16]. Matlabi et al. (2021) also reported that the combination of pectin and sodium alginate significantly improved the survival of Lactobacillus acidophilus La5 [21]. In the study by Shahmoradi et al. (2023), it was reported that dual coating of Lactobacillus acidophilus La5 and selenium with sodium caseinate and basil seed mucilage enhanced the survival of this probiotic bacterium under acidic laboratory conditions [22]. In the research by Oberoi et al. (2021), the effect of coating Lactobacillus rhamnosus with sodium alginate and its combination with sodium caseinate was also examined, and the coating process was found to improve the survival of the probiotic bacterium [23].

The goal of microencapsulation of probiotic bacteria is to stabilize and maintain their viability throughout the process, their use in food formulations, and their survival under human gastrointestinal conditions. This study was conducted to investigate the effect of the microencapsulation process on the survival rate of Lactobacillus acidophilus (La5) microencapsulated in a carbohydrate-protein composite matrix (sodium alginate-sodium caseinate) under simulated human gastrointestinal conditions.

2-Materials and methods

2-1- Preparation of microorganisms

We used a lyophilized pure culture of Lactobacillus acidophilus (La5) (Pro-Gen, Batch No.: R LAA5-165). To activate the bacteria and prepare a bacterial suspension, Lactobacillus acidophilus was inoculated into 10 mL of MRS[1] broth under aerobic conditions and incubated at 37°C for 24- 48 hours in a refrigerated incubator (Binder-KB 400). The turbidity of the medium indicated bacterial

[1] MRS: Man- Rogosa- Sharpe

[DOI: 10.22034/FSCT.22.159.144]

growth. Subsequently, the cells were separated using a refrigerated centrifuge (Z 326 K, HERMLE, Germany) at 3000 rpm and 4°C for 5 minutes and prepared for encapsulation [24].

2-2- Microencapsulation of microorganisms

Microencapsulation of Lactobacillus acidophilus (La5) was carried out using the emulsification technique with sodium alginate (Sigma-Aldrich, CAS No.: 90005-38-3) and sodium caseinate (Sigma-Aldrich, CAS No.: 90005-46-3) as encapsulation matrices. We tested five concentration levels, including single concentrations and combinations of the two materials: 25% sodium alginate with 75% sodium caseinate, 50% sodium alginate with 50% sodium caseinate, and 75% sodium alginate with 25% sodium caseinate. For this purpose, one part of a bacterial suspension containing 10^9–10^{10} CFU/g was mixed with four parts of sodium alginate and sodium caseinate (medium viscosity) using a magnetic stirrer. The resulting mixture was added dropwise to stirring vegetable oil (canola oil) to form a sodium alginate-sodium caseinate-oil emulsion. The emulsion was mixed at 900 rpm for 20 minutes using a centrifuge. To break the emulsion and form sodium alginate-sodium caseinate microcapsules, an external gelation method was employed. Specifically, 200 mL of 0.1 M calcium chloride solution was added dropwise to the sodium alginate/bacterial cell and sodium caseinate/bacterial cell emulsion while stirring at 100 rpm for 20 minutes. The mixture was then left undisturbed for 30 minutes to complete gelation and microcapsule formation. The resulting mixture was separated into two phases: an upper oil phase and a lower phase containing sedimented microcapsules. The oil phase was removed, and the lower phase was centrifuged at 600 rpm for 5 minutes to isolate the microcapsules. The microcapsules were then washed with peptone water. Finally, the microcapsules were dispersed in a calcium chloride solution and stored at 4°C until further use [25, 26].

2-3- Number of entrapped cells

Immediately after microencapsulation, 1 gram of the encapsulated cells was dissolved in 99 mL of sterile 1% sodium citrate solution (pH 6). The mixture was stirred at room temperature for 1 minute and left undisturbed for 10 minutes to ensure dissolution. Subsequently, the solution was plated on MRS agar under aerobic conditions and incubated at 37°C for 72 hours. The bacterial colonies were then enumerated [24].

2-4- Microencapsulation yield

To calculate encapsulation efficiency, the microcapsules were added to a peptone-saline solution (containing 1 g/L peptone and 8.5 g/L sodium chloride) at 37°C and shaken for 30 minutes to ensure complete mixing. The encapsulation efficiency (EY) was determined using Equation 1 and expressed as the number of colony-forming units (CFU/g) [27, 28].

$$EY = \frac{N}{N0} \times 100 \quad (1)$$

Where N represents the number of viable cells released from the capsules, and N_0 denotes the initial number of cells used in the encapsulation process.

2-5- Survival of microencapsulated bacteria in bile salt solution

1 gram of the microencapsulated cells was placed in a test tube containing 10 mL of sterile 0.6% bile solution at pH 8.25 and incubated at 37°C for 2 hours. At 60, 120, 180, and 240 minutes, the beads were washed and separated using 0.1% peptone water, and the enumeration of viable bacteria was performed as described in Section 2-3 [24].

2-6- Survival of microencapsulated bacteria in simulated gastrointestinal condition

1 gram of the microencapsulated cells was placed in a test tube containing 10 mL of sterile simulated gastric acid solution without pepsin (0.08 M HCl containing 0.2% NaCl, pH 1.55) and incubated at 37°C for 30, 60, 90, and 120 minutes. The samples were then exposed to 9 mL of intestinal fluid (0.05 M KH_2PO_4, pH

[DOI: 10.22034/FSCT.22.159.144]

7.43) with and without sterile bile salt and incubated at 37°C for 150, 180, 210, 240, and 300 minutes. A 1 mL aliquot of the dissolved beads was taken, and the enumeration of viable bacteria was performed as described in Section 2-3 [24].

2-7- Statistical analysis

Data analysis was performed using a factorial experiment based on a completely randomized design with three replications. In this design, the probiotic bacteria Lactobacillus acidophilus (La5) was encapsulated using coating agents at five concentration levels as described in Section 2-2, and the results were compared with the control sample (L-FC), which contained free and non-encapsulated bacteria. The optimal treatment was selected using an appropriate slicing method. Data analysis was conducted using SPSS software (version 21), and Duncan's test was applied at a 5% significance level for comparing group means.

3-Results and discussion

3-1- Number of encapsulated cells and encapsulation efficiency

The results for the number of viable cells after microencapsulation (log CFU/g) and encapsulation efficiency (as a percentage) are presented in Table 1.

Table 1- Number (log CFU/g) of surviving cells after microencapsulation and microencapsulation efficiency at different treatments

TREATMENT	SURVIVAL CELLS	MICROENCAPSULATION EFFICIENCY (PERCENTAGE)
L-FC	10.056 ± 0.123^{a}	0.000^{e}
L-SA	9.336 ± 0.06^{b}	92.847 ± 0.805^{ab}
L-SC	8.436 ± 0.289^{e}	83.881 ± 2.146^{d}
L-SA_1SC_3	8.703 ± 0.070^{cd}	86.551 ± 1.198^{cd}
L-SA_2SC_2	9.160 ± 0.141^{bc}	91.096 ± 1.197^{bc}
L-SA_3SC_1	9.673 ± 0.047^{ab}	96.197 ± 1.093^{a}

Different lowercase letters in each column indicate a significant difference at the level ($p<0.05$). The numbers in the table are the mean of three replicates ± standard deviation. L-Fc: Free cells, L-SA: Sodium alginate, L-SC: Sodium caseinate, L-SA_1SC_3: 25% Sodium alginate+75% Sodium caseinate, L-SA_2SC_2: 50% Sodium alginate+50% Sodium caseinate, L-SA_3SC_1: 75% Sodium alginate+25% Sodium caseinate.

Overall, we found significant differences in the various treatments ($p < 0.05$) regarding the number of viable cells remaining after coating and the coating efficiency. The initial number of viable cells before the coating process in the bacterial suspension was counted as 10.056 CFU/g. The number of trapped cells in the capsules for the different treatments indicated a low number of bacterial cells lost during the coating process. Based on the coating yield percentage, the L-SA_3SC_1 treatment about 96.19% showed the highest and the L-SC treatment about 83.88% the lowest trapping efficiency for viable cells within the microcapsules. This finding is in line with the study by Motalebi et al. (2021), which investigated the coating of Lactobacillus acidophilus (La5) and Bifidobacterium animalis (BB-12) using pectin and sodium alginate [21]. The coating efficiency affects physical characteristics such as stability, morphology, and release. High efficiency leads to better bioavailability and efficacy, whereas leakage of active compounds due to overloading or capsule degradation results in reduced efficiency, which may be caused by prolonged homogenization [29]. As shown in Table 1, the efficiency in different coating concentration levels was over 83%, indicating the suitability of the coatings for probiotic encapsulation. Lieu et al. (2020) compared the

[DOI: 10.22034/FSCT.22.159.144]

extrusion and emulsification coating methods for Lactobacillus acidophilus and reported an emulsification yield of 93.86 %, which aligns with the results of the present study [30].

A high cell loading range of 8.436 - 9.673 CFU/g and the high coating efficiency ranging from 83.88% to 96.19% for the coated bacteria showed significant differences between treatments ($p< 0.05$). The mild methods used during the coating process and the precision of the procedure were the reasons for the low bacterial loss during this stage, which is consistent with the findings of Krasaekoopt et al. (2004), who studied the effect of sodium alginate coating on the survival of probiotics [24]. Additionally, Zhang et al. (2015) confirmed the effectiveness of single-step and two-step emulsification methods for coating [28]. Overall, the results indicated that bacterial damage during the coating process using the emulsification method was minimal, making it a practical and suitable approach.

3-2- Survival of encapsulated cells in bile salt solution

The number of viable free and encapsulated microorganisms in the presence of bile salt at 0, 60, 120, 180, and 240 minutes is reported in Table 2. The viability of Lactobacillus acidophilus was assessed based on the time required to achieve a one-logarithmic reduction (D-value) from their initial population [24].

Table 2 – Number (log CFU/g) of encapsulated cells surviving against bile salt at different times

TREATMENT	TIME(MIN)					D-VALUE
	0	60	120	180	240	
L-FC	10.820±0.145^{a}	8.270±0.130^{c}	6.940±0.098^{c}	5.506±0.251^{c}	4.846±0.119^{d}	40.209±1.353^{b}
L-SA	10.140±0.220^{b}	9.266±0.128^{a}	8.646±0.120^{a}	7.303±0.112^{a}	6.630±0.190^{ab}	68.985±7.927^{a}
L-SC	9.137±0.122^{d}	8.210±0.147^{c}	7.556±0.920^{b}	6.710±0.292^{b}	5.953±0.140^{c}	75.720±6.142^{a}
L-SA_1SC_3	9.816±0.196^{bc}	8.616±0.137^{bc}	7.660±0.111^{b}	6.820±0.155^{b}	6.350±0.088^{bc}	69.361±3.645^{a}
L-SA_2SC_2	9.416±0.116^{cd}	8.273±0.085^{c}	7.490±0.790^{b}	6.733±0.087^{b}	6.093±0.179^{c}	72.572±6.179^{a}
L-SA_3SC_1	10.010±0.138^{b}	8.886±0.270^{ab}	7.753±0.145^{b}	7.213±0.080^{a}	6.903±0.086^{a}	77.503±5.288^{a}

Different lowercase letters in each column indicate a significant difference at the level ($p<0.05$). The numbers in the table are the mean of three replicates ± standard deviation. L-Fc: Free cells, L-SA: Sodium alginate, L-SC: Sodium caseinate, L-SA_1SC_3: 25% Sodium alginate+75% Sodium caseinate, L-SA_2SC_2: 50% Sodium alginate+50% Sodium caseinate, L-SA_3SC_1: 75% Sodium alginate+25% Sodium caseinate.

In this study, bile salt solution was used to determine whether sodium alginate and sodium caseinate coatings enhance the survival of probiotic cells in an environment resembling the gastrointestinal system. A significant difference was observed between free cells and various treatments ($p<0.05$) (Table 2). While all treatments showed a decrease over time as expected, this decrease was less pronounced in the coated treatments compared to the free cells. For free bacteria, the highest survival rate against bile salt was observed at the initial time, with the lowest survival rate at minute 240, showing a decreasing trend with a D-value of 40.209 for the control sample (L-FC). Among the coated treatments, the highest survival over the specified time intervals was observed for the L-SA_3SC_1 treatment, with a D-value of 77.503, while the lowest survival rate was found in the L-SA treatment, with a D-value of 68.985 Therefore, coating had a significant positive effect in increasing the survival of

[DOI: 10.22034/FSCT.22.159.144]

Lactobacillus acidophilus against bile salt over the designated periods.

Bile salt absorption by probiotics induces an ion exchange reaction. In the sodium alginate-sodium caseinate membrane, an insoluble complex forms between sodium alginate, sodium caseinate, and bile salts, which limits the diffusion of bile salts into the microcapsules, thereby protecting the coated cells from bile salt exposure. As the isoelectric point of sodium caseinate is 6.4, those molecules carry positive charges when exposed to the gastrointestinal environment, and thus tend to repel each other, leading to the dissolution of the microcapsules and loss of bacterial protection. In contrast, the carboxyl groups of sodium alginate form strong hydrogen bonds with the amide and carboxyl groups of sodium caseinate molecules, enhancing the strength of the polymeric network and preventing the dissolution of the microcapsules, thereby protecting the cells within the polymeric matrix [31]. Kim et al. (2008) reported that coating significantly enhanced the survival of Lactobacillus acidophilus (ATCC43121) against bile salts, which is consistent with the results of this study [32]. In the studies of Kailasapathy (2006) and Chandramouli et al. (2004) on the effect of coating on the survival of probiotics with bile salt concentrations ranging from 1% to 3%, it was reported that the survival of coated probiotics was higher than that of free probiotics [33, 34].

3-3- Survival of microencapsulated cell in simulated gastrointestinal condition

Before Lactobacillus acidophilus can be utilized as a functional product with positive health effects, it must survive passage through the gastric environment and remain viable in sufficient numbers to proliferate in the colon. In this study, the survival of free and encapsulated bacteria was evaluated during incubation for 0–120 minutes in a simulated gastric solution and 150–300 minutes in a simulated intestinal solution, both with and without bile salt [24].

3-3-1- Survival of encapsulated cells in simulated gastric juice

The number of free and microencapsulated viable microorganisms surviving in the simulated gastric environment at 0, 30, 60, 90, and 120 minutes is reported in Table 3. The bacterial viability of Lactobacillus acidophilus was calculated based on the time required to reduce one logarithmic cycle (D-value) from their initial population [24].

Table 3 - Number (log CFU/g) of microencapsulated cells surviving against gastric acid

TREATMENT	TIME(MIN)					D-VALUE
	0	30	60	90	120	
L-FC	10.486 ±0.170^{a}	7.933± 0.061^{b}	5.870±0.140^{c}	4.893±0.257^{c}	2.976±0.180^{d}	16.001±0.744^{c}
L-SA	9.956±0.061^{bc}	8.863±0.145^{a}	7.173±0.085^{b}	6.336±0.132^{a}	5.176±0.115^{ab}	25.120±0.764^{a}
L-SC	9.333±0.182^{d}	8.543±0.462^{ab}	6.846±0.070^{b}	5.541±0.120^{bc}	4.076±0.159^{c}	22.831±0.352^{b}
L-SA_1SC_3	9.990±0.125^{b}	9.106±0.142^{a}	8.036±0.015^{a}	6.103±0.159^{a}	5.273±0.092^{a}	25.445±0.384^{a}
L-SA_2SC_2	9.543±0.070^{cd}	8.810±0.174^{a}	7.136±0.055^{b}	6.266±0.128^{a}	5.036±0.140^{ab}	26.712±0.536^{a}
L-SA_3SC_1	9.323±0.085^{d}	8.470±0.045^{ab}	7.070±0.121^{b}	5.846±0.081^{ab}	4.863±0.095^{b}	26.912±0.514^{a}

Different lowercase letters in each column indicate a significant difference at the level ($p<0.05$). The numbers in the table are the mean of three replicates ± standard deviation. L-Fc: Free cells, L-SA: Sodium alginate, L-SC: Sodium caseinate, L-SA_1SC_3: 25% Sodium alginate+75% Sodium caseinate, L-SA_2SC_2: 50% Sodium alginate+50% Sodium caseinate, L-SA_3SC_1: 75% Sodium alginate+25% Sodium caseinate.

Based on the results obtained from the comparison of mean data in the survival test of encapsulated microorganisms under simulated gastric acid conditions, significant differences were observed between free cells and various treatments ($p<0.05$). After 120 minutes,

[DOI: 10.22034/FSCT.22.159.144]

survival decreased across all treatments; however, survival rates were significantly higher in encapsulated samples compared to the control treatment ($p<0.05$). Due to low resistance to acidic conditions, free Lactobacillus acidophilus cells exhibited no survival in the presence of gastric juice, with their count reducing to 2.976 CFU/g within 120 minutes. In contrast, under similar conditions and duration, the encapsulated treatments showed survival rates ranging from 4.076 to 6.336 CFU/g. Among the treatments, the highest survival was observed in the L-SA_3SC_1 treatment, with a D-value of 26.912, while the lowest survival was noted in free cells (L-FC) with a D-value of 16.001. The superior survival trend of microcapsules can be attributed to the lack of direct contact between the cells and the acidic environment, as well as the delayed diffusion of gastric juice. This phenomenon leads to enhanced survival and improved viability of probiotics, which is the primary goal of encapsulation. In this study, the enhanced survival of encapsulated cells in the simulated gastric environment is likely explained by the synergistic effect of sodium alginate and sodium caseinate, along with the pre-adaptation of bacterial cells in low-pH caseinate gels [35].

Similar findings were reported by Shamoradi et al. (2023), who demonstrated that sodium caseinate encapsulation improved the survival of encapsulated Lactobacillus acidophilus compared to free cells [22]. Additionally, Oberoi et al. (2021) and Holkem et al. (2017) also highlighted the positive impact of sodium alginate encapsulation on the survival of probiotics under gastrointestinal acidic conditions [23, 26].

3-3-2- Survival of encapsulated cells in simulated intestinal juice with and without bile salt

The number of free and microencapsulated viable microorganisms surviving in the simulated intestinal environment in the presence of bile salt at 150, 180, 210, 240, and 300 minutes is reported in Table 4. The bacterial viability of Lactobacillus acidophilus was calculated based on the time required to reduce one logarithmic cycle (D-value) from their initial population [24].

Table 4– Number (log CFU/g) of microencapsulated cells surviving against the simulated intestinal environment with bile salt

TREATMENT	TIME(MIN)					D-VALUE
	150	180	210	240	300	
L-FC	2.603±0.165^{d}	1.43±0.160^{d}	0.923±0.188^{d}	0.566±0.073^{d}	0^{d}	46.220±2.974^{b}
L-SA	4.813±0.045^{a}	4.043±0.065^{b}	3.523±0.090^{b}	3.556±0.187^{a} b	3.036±0.060^{a} b	67.573±1.775^{a} b
L-SC	3.580±0.105^{c}	3.246±0.060^{c}	2.826±0.145^{c}	2.426±0.135^{c}	2.156±0.176^{c}	86.024±14.200^{a}
L-SA_1SC_3	5.046±0.065^{a}	4.573±0.140^{a}	4.256±0.068^{a}	3.826±0.040^{a}	3.426±0.173^{a}	74.629±7.690^{a} b
L-SA_2SC_2	4.423±0.450^{b}	3.973±0.073^{b}	3.653±0.132^{b}	3.280±0.110^{b}	2.866±0.135^{b}	84.146±8.964^{a}
L-SA_3SC_1	4.750±0.140^{a} b	4.550±0.079^{a}	4.116±0.090^{a}	3.833±0.102^{a}	3.383±0.030^{a}	88.184±7.078^{a}

Different lowercase letters in each column indicate a significant difference at the level ($p<0.05$). The numbers in the table are the mean of three replicates ± standard deviation. L-Fc: Free cells, L-SA: Sodium alginate, L-SC: Sodium caseinate, L-SA_1SC_3: 25% Sodium alginate+75% Sodium caseinate, L-SA_2SC_2: 50% Sodium alginate+50% Sodium caseinate, L-SA_3SC_1: 75% Sodium alginate+25% Sodium caseinate.

[DOI: 10.22034/FSCT.22.159.144]

The number of viable free and encapsulated microorganisms in a simulated intestinal environment without bile salt, as well as under similar conditions with bile salt, is reported in Table 5. The viability of Lactobacillus acidophilus was determined based on the time required to achieve a one-logarithmic reduction (D-value) from their initial population [24].

Table 5- Number (log CFU/g) of microencapsulated cells surviving against the simulated intestinal environment without bile salt

TREATMENT	TIME(MIN)					D-VALUE
	150	180	210	240	300	
L-FC	2.850±0.069 d	2.430±0.070 e	2.120±0.124 d	0.920±0.600 c	0.296±0.145 d	47.037±1.678 d
L-SA	4.956±0.037 a	4.436±0.075 b	3.753±0.135 b	3.326±0.136 b	3.123±0.04 b	65.508±2.339 c
L-SC	3.830±0.110 c	3.573±0.097 d	3.153±0.106 c	2.900±0.045 b	2.090±0.953 c	69.618±8.337 b c
L-SA_1SC_3	4.863±0.080 a	4.526±0.085 b	3.986±0.055 b	3.636±0.125 ab	3.243±0.08 b	74.419±6.066 b c
L-SA_2SC_2	4.606±0.015 b	4.153±0.060 c	3.750±0.090 b	3.433±0.115 ab	3.146±0.102 b	82.995±5.339 b
L-SA_3SC_1	4.916±0.051 a	4.780±0.026 a	4.593±0.083 a	4.263±0.104 a	4.006±0.05 a	131.941±3.783 a

Different lowercase letters in each column indicate a significant difference at the level ($p<0.05$). The numbers in the table are the mean of three replicates ± standard deviation. L-Fc: Free cells, L-SA: Sodium alginate, L-SC: Sodium caseinate, L-SA_1SC_3: 25% Sodium alginate+75% Sodium caseinate, L-SA_2SC_2: 50% Sodium alginate+50% Sodium caseinate, L-SA_3SC_1: 75% Sodium alginate+25% Sodium caseinate.

Based on the results obtained from Tables (4) and (5), the comparison of mean data from the test of the number of microencapsulated viable microorganisms in the gut environment with and without bile salts shows a significant difference between free cells and treatments ($p<0.05$). Additionally, over time, the viability of all treatments decreased. The highest viability in the treatments was observed in the L-SA_3SC_1 treatment, with a D-value of 131.941 in the gut environment without bile salts and 88.184 in the presence of bile salts. The lowest viability was found in the free cells (L-FC), with a D-value of 47.037 in the gut environment without bile salts and 46.220 in the presence of bile salts. Therefore, microencapsulation under gut conditions can enhance bacterial viability. Furthermore, when comparing conditions with and without bile salts, treatments in the gut environment without bile salts exhibited higher viability than in the presence of bile salts.

In the study by Zeashan et al. (2019), investigating the encapsulation on probiotic survival, free cells showed a rapid decrease compared to encapsulated cells. Sodium alginate was also used in this study, which improved probiotic survival under simulated gastrointestinal conditions [36]. Oberoi et al. (2021) examined the effect of encapsulation on Lactobacillus rhamnosus and reported that the use of sodium alginate combined with xanthan gum could offer protective effects in the gut environment [23]. Additionally, in a study by Mokarram et al. (2009), after incubating encapsulated strains, the D-value of Lactobacillus acidophilus and Lactobacillus rhamnosus with sodium alginate in simulated gastric (60 minutes) and intestinal (7.25 pH, 2 hours) environments were reported as 30.84 CFU/g and 26.43 CFU/g, respectively, whereas for free cells, these values were 16.19 CFU/g and 15.13 CFU/g [37]. In the study by Krasaekoopt et al. (2004), encapsulated

[DOI: 10.22034/FSCT.22.159.144]

Lactobacillus acidophilus cells in sodium alginate capsules, after consecutive incubation in simulated gastric and intestinal juices with or without bile salts, survived better than free cells, and the chitosan coating further enhanced cell viability compared to other coatings [24]. The results of the present study align with all the aforementioned studies.

4-Conclusion

In the consumption of probiotic food products, in addition to ensuring a standard number of probiotic bacteria reach the intestine, the primary condition for achieving the desired health-promoting effects is the survival of the probiotic bacteria until reaching the end of the gastrointestinal tract. To achieve this, the microbial strain must withstand the acidic conditions of the gastric juice and bile salts in the small intestine during digestion and processing. The significance and diversity of probiotic products, as well as the success of microencapsulation methods in enhancing probiotic viability, have increased attention on this approach. We demonstrated that Lactobacillus acidophilus La5, in both free and microencapsulated forms with sodium alginate and sodium caseinate through emulsification significantly enhances the survival rate of this probiotic compared to the free-form. A statistically significant difference in the survival rate of microencapsulated strains versus free bacteria was observed in all tests. Additionally, the combination of sodium alginate and sodium caseinate as coating agents played a substantial role in the successful microencapsulation process. Based on these results, it can be concluded that microencapsulation positively affects the survival of Lactobacillus acidophilus when exposed to a simulated gastrointestinal environment.

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[DOI: 10.22034/FSCT.22.159.144]

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مقاله علمی_پژوهشی

ریزپوشانی لاکتوباسیلوس اسیدوفیلوس La_5 در بستر آلژینات سدیم و کازئینات سدیم و قابلیت زنده‌مانی آن در شرایط شبیه‌سازی شده گوارشی

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۳/٤/۳۱

تاریخ پذیرش: ۱۴۰۳/٦/۲۱

کلمات کلیدی:

ریزپوشانی

پروبیوتیک،

لاکتوباسیلوس اسیدوفیلوس،

آلژینات سدیم،

کازئینات سدیم

DOI:10.22034/FSCT.22.159.144.

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چکیده

زنده‌مانی پروبیوتیک‌ها در محیط‌های حساس به‌خصوص شرایط گوارشی اهمیت زیادی دارد. در این تحقیق از تکنیک ریزپوشانی به‌عنوان روشی برای افزایش امکان زنده‌مانی پروبیوتیک‌ها (لاکتوباسیلوس اسیدوفیلوس La_5) در شرایط شبیه‌سازی شده گوارشی استفاده گردید. باکتری‌های مذکور به روش امولسیون‌سازی در بستر آلژینات سدیم و کازئینات سدیم در پنج سطح غلظتی به‌صورت تک غلظتی و ترکیب دو ماده میکروانکپسوله شدند و از نظر تعداد سلول زنده مانده، بازده انکپسولاسیون، زنده‌مانی در برابر نمک صفرا، زنده‌مانی در برابر اسید معده و مایع روده‌ای با و بدون نمک صفرا مورد بررسی قرار گرفتند. با توجه به نتایج به دست آمده از مقایسه میانگین داده‌ها در تمامی آزمون‌ها بین تیمارهای مختلف اختلاف معناداری وجود داشت ($p<0/05$)؛ به‌طوری که زنده‌مانی سلول‌های آزاد در شرایط گوارشی به شدت کاهشی بود اما میکروانکپسولاسیون به‌عنوان یک عامل محافظتی عمل نموده و زنده‌مانی سویه‌های میکروانکپسوله بیشتر از سلول‌های آزاد بود. از طرفی ترکیب آلژینات سدیم و کازئینات سدیم به عنوان پوشش توانست مقاومت باکتری را در برابر شرایط گوارشی به‌طور معناداری افزایش دهد($p<0/05$). در بین تیمارها، سلول‌های آزاد (L-FC) کمترین زنده‌مانی در برابر محیط گوارشی را از خود نشان داد به‌طوری که در آزمون زنده‌مانی در محیط روده با نمک صفرا پس از ۳۰۰ دقیقه سلول زنده‌ای وجود نداشت اما تیمار با پوشش ۷۵٪ آلژینات سدیم و ۲۵٪ کازئینات سدیم ($L\text{-}SA_3SC_1$) بالاترین بازده را در فرآیند میکروانکپسولاسیون داشته و بهترین اثر محافظتی در برابر نمک صفرا، اسید معده و مایع روده‌ای را از خود نشان داد. بنابراین می‌توان نتیجه گرفت میکروانکپسولاسیون به روش امولسیون‌سازی با استفاده از ترکیب آلژینات سدیم و کازئینات سدیم می‌تواند در افزایش زنده ماندن باکتری لاکتوباسیلوس اسیدوفیلوس موثر باشد

[DOI: 10.22034/FSCT.22.159.144]